627

ASSIMILATION OF DAIRY WASTES BY ACTIVATED SLUDGE

II. The Equation of Synthesis and Rate of Oxygen Utilization *

By Sam R. Hoover and Nandor Porges

Eastern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Dept. of Agriculture, Philadelphia, Pa.

In previous investigations, the authors (3)(4) have studied the biochemical oxidation of milk solids by activated sludge. The primary reaction was shown to be

$$\text{Milk solids} + O_2 \rightarrow \text{bacterial cells} + CO_2 + H_2O. \quad (1)$$

The total cell tissue synthesized was found by analysis of the protein and carbohydrate contents of the cells to be equal to 47 per cent of the protein and carbohydrate in the milk solids (3). On a weight basis, the yield of cell material was slightly higher; about 52 per cent, for the cells contain about 9 per cent ash. These results were obtained in continuous flow experiments after steady-state operation had been attained. In a later study of this assimilation reaction, the oxygen consumed and carbon dioxide produced were measured manometrically (2). Simultaneous chemical analysis of the amount of substrate remaining was employed to determine when the substrate was exhausted. It was established that the substrate was completely consumed when about 32 to 40 per cent of the oxygen required for complete oxidation had been used. Conversely, an assimilation of 60 per cent was indicated by these data.

The respiratory quotient (R.Q.), which is the ratio of the volume of CO_2 produced to that of oxygen consumed, was determined. The R.Q. of a biochemical reaction is of value because it gives a direct measure of the conversion of atmospheric oxygen to carbon dioxide. If the substrate and non-volatile reaction products are known, the equation of the occurring reaction can be established. For example, the complete oxidation of ethyl alcohol to CO_2 and water has an R.Q. of 0.67, whereas the oxidation of the same compound to acetic acid requires oxygen and produces no CO_2; the R.Q. is, consequently, zero. For the assimilation of milk constituents by cells, the R.Q. was 1.00 for casein, 1.04 for lactose, and 1.03 for skim milk.

Composition of Cells

For a detailed consideration of the system, however, a chemical analysis of the synthesized cell tissue was required. The previous analyses of the cells had been for carbohydrate, protein, and ash content. The oxygen, carbon, hydrogen, nitrogen, and ash contents of the cells were determined, therefore, so that the empirical composition of the organisms could be established. As far as is known, these are the first direct determinations of oxygen in microorganisms. The percentage of each element present was calculated to a molar basis by dividing the determined value by the respective atomic weight. As shown in Table I, $C_5H_7NO_2$ is a close approximation of the resultant composition.

* Presented in part at the meeting of the American Assn. for Advancement of Science; Philadelphia, Pa.; Dec. 27, 1951.

Part I of this series appeared in This Journal, 23, 2, 167 (Feb., 1951).

TABLE I.—Empirical Composition of Sludge Organisms

Constituent	Weight (%)	Atomic Ratio	
		%/At. Wt.	%/At. Wt.
C	47.26	3.94	4.89
H	5.69	5.65	7.02
N	11.27	0.805	(1.0)
O	27.0	1.69	2.09
Ash	8.61	—	—
Total	99.83	—	—

It is realized that a microbial cell is an organized system of almost infinite complexity; this empirical formula expresses only the statistical average proportions of the major atoms of the organic constituents. For purposes of calculation, this $C_5H_7NO_2$ unit has a "mole wt." of 113. Corrected for the ash content of 8.6 per cent the weight of a "mole" of cells is practically 124 atomic weight units. This figure will be of value in subsequent calculations.

Cell Synthesis from Lactose

The conversion of carbohydrate to cell tissue requires energy, which is obtained by oxidation of a portion of the carbohydrate. Because no by-products are produced in significant quantities by this aerobic oxidation, the energy-yielding step must be the complete oxidation of lactose to carbon dioxide and water.

$$C_{12}H_{22}O_{11} \cdot H_2O + 12\,O_2 \rightarrow 12\,CO_2 + 12\,H_2O \quad (2)$$

For convenience, the repeating unit

$$\begin{array}{c} | \\ \text{HCOH} \\ | \end{array}$$

which requires one molecule of oxygen for its oxidation, is used.

$$(CH_2O) + O_2 \rightarrow CO_2 + H_2O \quad (3)$$
$$R.Q. = 1/1 = 1.0$$

The minimum equation for the conversion of carbohydrate to cell tissue, if ammonia is used as the source of nitrogen, is:

$$5\,(CH_2O) + NH_3 \rightarrow C_5H_7NO_2 + 3\,H_2O \quad (4)$$

Eq. 4 is balanced without oxygen consumption or CO_2 evolution. According to the manometric data, about 37 per cent of the oxygen required for completely oxidizing the sugar was used during this assimilation. Therefore, there must be added to Eq. 4 the oxidation of three units of sugar, according to Eq. 3, to give

$$3\,O_2 + 8\,(CH_2O) + NH_3 \rightarrow C_5H_7NO_2 + 3\,CO_2 + 6\,H_2O \quad (5)$$
$$R.Q. = 3/3 = 1.0$$

Three-eighths, or 37.5 per cent, of the amount of oxygen required for complete oxidation is consumed. The experimental R.Q. of 1.04 agrees well with the theoretical value of 1.0. The data on yield of cells by weight are also consistent: 240 atomic weight units, 8 (CH_2O), are used up, and 124 units of cells are produced; 124/240 = 52 per cent yield by weight. The experimental data show a yield by weight of slightly greater than 50 per cent. The latter data are for the assimilation of dry skim milk, however, and not for lactose alone.

Cell Synthesis from Casein

Similar equations have been established for the oxidation and assimilation of casein. The four elements tabulated were determined directly. Both sulfur and phosphorus occur in casein; about 0.8 per cent of each. This content, calculated to the molar basis used, would equal only 0.05 atom per mole and therefore can be neglected. The "mole" weight of $C_8H_{12}N_2O_3$ is 184. This value, obtained from Table II, will be referred to later.

The equation for complete oxidation of casein is readily established if an assignment of the nitrogen can be made. It is probable that nitrogen is not oxidized until all the carbon has been oxidized; therefore, Eq. 6 was

TABLE II.—Composition of Casein

Constituent	Weight (%)	Atomic Ratio	
		%/At. Wt.	%/At. Wt.
C	52.85	4.40	8.15
H	6.48	6.43	11.9
N	15.12	1.08	(2.0)
O	24.76	1.55	2.87
Total	99.21	—	—

written on the basis of ammonia release.

$$C_8H_{12}N_2O_3 + 8\ O_2 \rightarrow 8\ CO_2 + 2\ NH_3 + 3\ H_2O \quad (6)$$
$$R.Q. = 8/8 = 1.0$$

Thus, by comparing Eq. 6 with Eq. 2, it can be seen that complete oxidation of one carbon atom from either lactose or casein requires one O_2 and produces one CO_2.

The minimum equation for the assimilation of casein into cell tissue may be written:

$$C_8H_{12}N_2O_3 + 3\ O_2 \rightarrow C_5H_7NO_2 + NH_3 + 3\ CO_2 + H_2O \quad (7)$$
$$R.Q. = 3/3 = 1.0$$

Eq. 7 is not rigorous, for it is based on the assumption that one-half the nitrogen is assimilated and one-half released as free ammonia. There is qualitative evidence of ammonia release when cells are grown on casein alone. Ammonia is apparent in the gas phase, and the pH of the solution is increased, but the amount of ammonia produced has not been determined. The minimum equation (Eq. 7) has an R.Q. of 1.0, the same as that observed experimentally. The amount of oxygen required for the assimilation (compare Eqs. 6 and 7) is three-eighths that for complete oxidation. The almost exact equivalence of the amount of oxygen required and of the R.Q. for the assimilation of lactose and casein into cell tissue is explained by this series of equations.

Cell Synthesis from Skim Milk Solids

The assimilation equations (Eqs. 5 and 7), each of which produces one "mole" of cells, may be compared further. Eq. 5 requires 8 (CH_2O) units or 240 atomic weight units of sugar, whereas Eq. 7 requires 184 units of casein. The proportion of 240:184 is essentially that of the relative proportions of lactose to casein in skim milk, 51:37, and the two equations may be summed directly, as follows:

$$8\ (CH_2O) + 3\ O_2 + NH_3 \rightarrow C_5H_7NO_2 + 3\ CO_2 + 6\ H_2O \quad (5)$$
$$C_8H_{12}N_2O_3 + 3\ O_2 \rightarrow C_5H_7NO_2 + 3\ CO_2 + H_2O + NH_3 \quad (7)$$
$$8\ (CH_2O) + C_8H_{12}N_2O_3 + 6\ O_2 \rightarrow 2\ C_5H_7NO_2 + 6\ CO_2 + 7\ H_2O \quad (8)$$
$$R.Q. = 1.0$$

The summation equation requires six-sixteenths, or 37.5 per cent, of the oxygen needed for complete oxidation; the ammonia required for the carbohydrate assimilation is furnished by the protein. Thus, no pH change due to ammonia release would be expected. The pH does not change when skim milk is the substrate (3). The R.Q. of 1.0 for the assimilation is in excellent agreement with the experimental value of 1.03.

The amount of cell tissue produced from a given amount of skim milk, according to Eq. 8, is $\frac{2 \times 124}{240 + 184} = 58.5$ per cent. This value is slightly greater than the 52 per cent observed earlier (2) (3). The difference arises, apparently, from the failure of the continuous-flow experiments to effect the complete conversion of skim milk to cells, carbon dioxide, and water as required by Eq. 8 and observed in the manometric experiments. Thus, it is probable that in continuous-flow or fill-and-draw experiments the maximum amount of cell tissue obtainable experimentally will be 52 to 55 per cent. If conditions do not permit the most rapid growth, the yield of cell tissue will be lower, for the cells will continue to oxidize (at a lower rate) the sugar and protein essentially according to Eqs. 2 and 6, with an accompanying increase in the proportion of the substrate oxidized to CO_2 and H_2O.

Endogenous Respiration

The reactions expressed by Eqs. 1 to 8 are those of the synthetic reaction. The cells produced in this reaction have what is called an endogenous respiration; that is, an oxidation of their own tissue for energy. It is analogous to the basal metabolism in humans.

$$C_5H_7NO_2 + 5\ O_2 \rightarrow 5\ CO_2 + NH_3 + 2\ H_2O \quad (9)$$
$$R.Q. = 1.0\ *$$

* The low Q_{O_2} makes determination of R.Q. difficult, for a CO_2 production of 200 to 300 μL. is required for an accurate R.Q. determination. However, duplicate values of 0.95 were obtained in the most satisfactory experiment.

Eq. 9 has an R.Q. of 1.0, which can be determined manometrically by measuring the rate of oxygen consumption of the sludge without added substrate. A Q_{O_2}, or rate of oxygen consumption, of about 12 μL. per mg. per hr. was reported previously. The Q_{O_2} reported for Eq. 8 was 50 to 150, about 10 times the endogenous respiration (2).

Polarographic Determination of the Rate of Oxidation

Direct determination of dissolved oxygen by polarographic measurements may be used to determine (a) the relative rate of oxidation by the synthetic reaction to that of endogenous respiration, and (b) the effect of low concentration of oxygen on these two rates. Oxygen depletion rates of bacterial sludge for various concentrations of nutrient were measured by a modification of the method

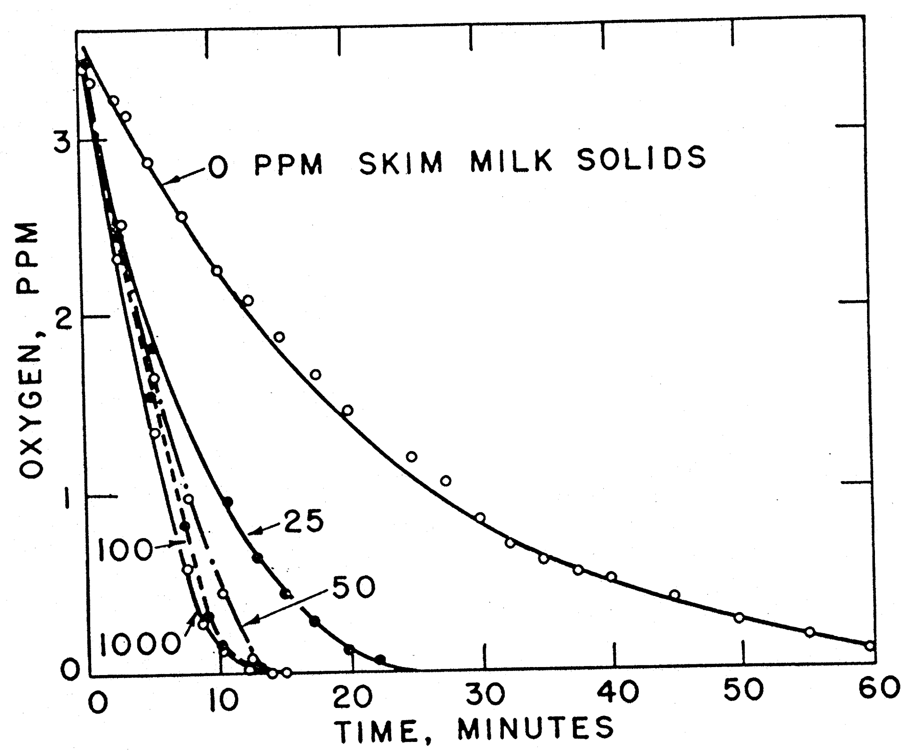

FIGURE 1.—Oxygen removed from solutions containing the indicated amounts of skim milk solids by activated sludge under anaerobic conditions.

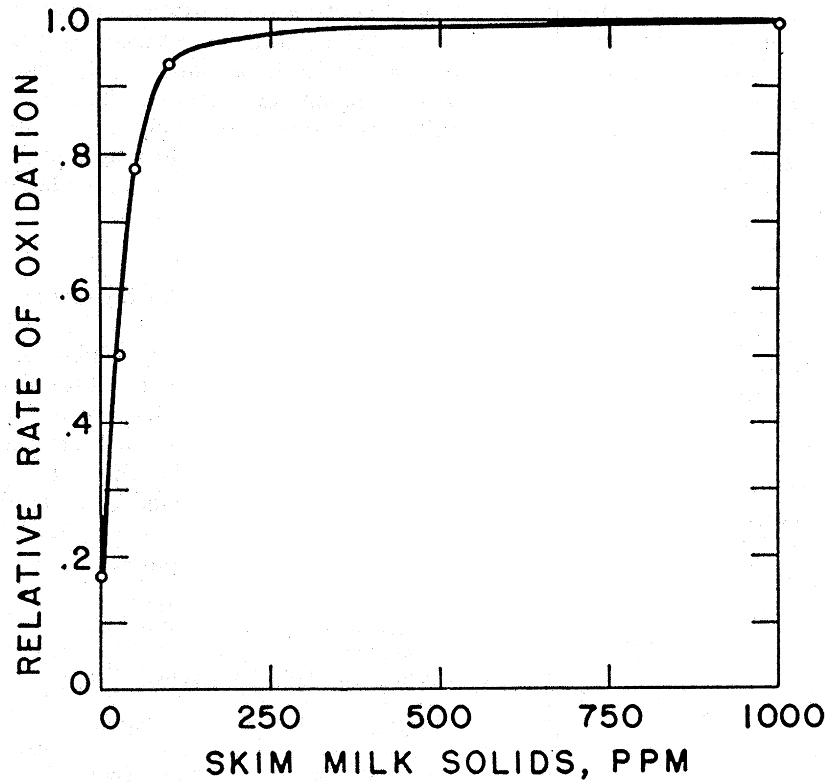

FIGURE 2.—Rate of oxidation by activated sludge as a function of the concentration of skim milk solids. The time required to reduce the concentration of oxygen to 0.5 p.p.m. for the maximum substrate concentration is taken as unity.

of Hixon and Gaden (1). In the present studies, oxygen depletion rates of active bacterial sludges were determined by recording continuous polarograms of the oxygen remaining in solution.† Measurements of oxygen depletion were made at 0, 25, 50, 100, and 1,000 p.p.m. of nutrient.

The results show a rapid and relatively constant rate of oxidation of skim milk solids. The curves of oxygen depletion (Figure 1) can be interpreted more satisfactorily when plotted as in Figures 2 and 3. The rate of oxidation as a function of substrate concentration is approximated by determining the time required to reduce the oxygen tension to 0.5 p.p.m. The most rapid rate of depletion at 1,000 p.p.m. is taken as unity. Any other arbitrary end point could have been selected without affecting the results signifi-

† Use of a sealed, modified Lingane H-cell (5) with nitrogen-filled head space prevented absorption of atmospheric oxygen by the sludge during the oxygen measurements. The oxygen remaining in solution was measured continuously at an applied potential of − 0.5 volts *vs.* S.C.E., with the cell held at 25 ± 0.1° C. The dissolved salts in the substrate served as the supporting electrolyte. The sludge was dispersed by high-speed agitation so that it would not settle rapidly before it was transferred to the H-cell. The sludge was aerated, and the nutrient was added and mixed by further air bubbling. After this, the air in the head space was replaced with nitrogen, the cell was sealed, and a continuous polarogram was recorded until the height of the oxygen wave attained a constant minimum value. These wave heights were calibrated with solutions containing known quantities of dissolved oxygen, and since the polarogram abscissae were expressed in minutes, oxygen depletion rate constants, p.p.m. O_2 per minute, were obtained directly from the slope of these waves.

cantly. The rate of assimilation by Eq. 8 is essentially constant above 100 p.p.m. of added skim milk. The time required to oxidize the waste, therefore, is directly proportional to the amount added in this range. It has been previously shown by Warburg respirometer studies that 1,000 p.p.m. are assimilated in about 6 hr.; 500 p.p.m. would then be assimilated in 3 hr., and other amounts in similar proportion. Such calculations assume that a sufficient amount of oxygen is supplied. In these experiments, the assimilation reaction proceeded at 6 times the rate of endogenous respiration shown by the control without added substrate.

Figure 3 shows the rate of oxidation of 100 p.p.m. skim milk solids (from Figure 1) as a function of oxygen concentration. Only below about 0.5 p.p.m. O_2 does the rate fall off appreciably because of lack of oxygen. The absolute rate of oxygen consumption is of practical importance; oxygen is removed from the solution at a rate of 0.35 p.p.m. per minute. The difficulty encountered in supplying oxygen at such a rate is one of the major problems in the aerobic treatment of dairy wastes.

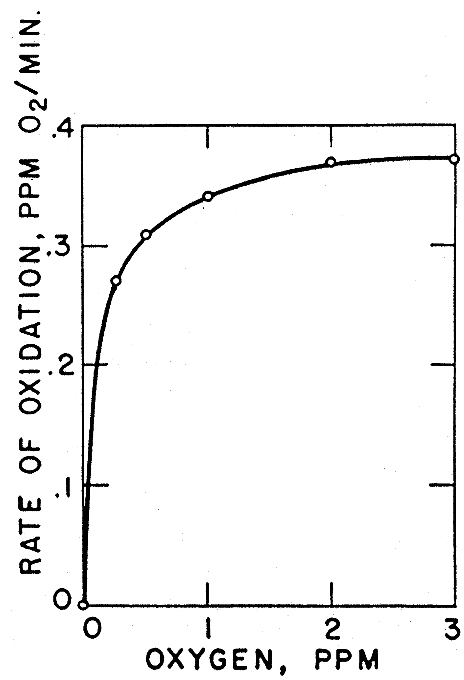

FIGURE 3.—Rate of oxidation of 100 p.p.m. skim milk solids as a function of oxygen concentration. The rate is taken from the slope of the curve for this concentration in Figure 1.

Discussion

Practical implications of the oxygen-depletion data are discussed above. Additional considerations, which are believed to be of importance in the design and operation of dairy waste disposal plants, may be derived from the equations established in this paper. The following calculations are based on 1 lb. of skim milk solids in 1,000 lb. of water; that is, 1,000 p.p.m.

Assimilation reaction (Eq. 8):	
O_2 required (%) for complete oxidation	37.5
Time required for assimilation (hr.)	6
Oxygen required for assimilation (lb.)	0.45
Rate of oxygen utilization (lb./hr.)	0.075
Endogenous respiration (Eq. 9):	
O_2 required (%) for complete oxidation	62.5
Time required for oxidizing 500 p.p.m. cells produced according to Eq. 8 (hr.)	30
Oxygen required for oxidizing cells (lb.)	0.75
Rate of oxygen utilization (lb./hr.)	0.025

The assimilation reaction is assumed to be 10 times as rapid as endogenous respiration. This relationship was obtained several times in a previous study. If the sludge were less active than is assumed, the assimilation reaction would be slower, perhaps requiring 8 hr. for completion. The rate of endogenous respiration is relatively constant, about 12 μL. per mg. per hr. (2), despite variations in the rate of substrate oxidation caused by changes in activity of the sludge. If about 2,500 p.p.m. sludge solids were carried in an aerator, the time for completion of the endogenous reaction would not differ appreciably, but the rate of oxygen utilization would increase proportionately (5-fold) and more oxygen must be supplied. In these experiments, the solids content of the sludge at the start was about 500 p.p.m.

Summary

Chemical equations for conversion of lactose, casein, and skim milk solids to activated sludge are established.

A similar equation is derived for the endogenous respiration of the sludge. The rate of these reactions is measured by polarographic determination of oxygen depletion. The various lines of evidence combine to give a quantitative description of the biochemical processes occurring in the oxidation of dairy waste by activated sludge.

Acknowledgment

The technical assistance of Janet B. Pepinsky and Lenore B. Jasewicz in determination of R.Q. values and of Mary Jane Welsh and Ruth Kelly in the elemental analyses on which the calculations are based is gratefully acknowledged. The authors are also grateful for the assistance of Constantine Ricciuti, who developed the necessary polarographic procedures for the continuous measurement of oxygen depletion of an oxygen-saturated activated sludge.

The study reported herein was made under the Research and Marketing Act of 1946.

References

1. Hixon, A. W., and Gaden, E. L., Jr., "Oxygen Transfer in Submerged Fermentation." *Ind. Eng. Chem.*, **42**, 1792 (1950).
2. Hoover, S. R., Jasewicz, L., Pepinsky, J. B., and Porges, N., "Assimilation of Dairy Wastes by Activated Sludge." THIS JOURNAL, **23**, 2, 167 (Feb., 1951).
3. Hoover, S. R., and Porges, N., "Treatment of Dairy Waste by Aeration. II. Continuous Aeration Studies." Proc. 5th Ind. Waste Conf., Purdue Univ., p. 137 (July, 1950).
4. Porges, N., Pepinsky, J. B., Hendler, N. C., and Hoover, S. R., "Biochemical Oxidation of Dairy Wastes. I. Methods of Study." THIS JOURNAL, **22**, 3, 318 (Mar., 1950).
5. Willits, C. O., Ricciuti, C., Knight, H. B., and Swern, D., "Polarographic Studies of Oxygen-Containing Organic Compounds. I. Functional Groups of Autoxidation Products." *Anal. Chem.* (in press).